

## GRISORIXIN, AN IONOPHOROUS ANTIBIOTIC OF THE NIGERICIN GROUP

II. CHEMICAL AND STRUCTURAL STUDY OF GRISORIXIN  
AND SOME DERIVATIVES

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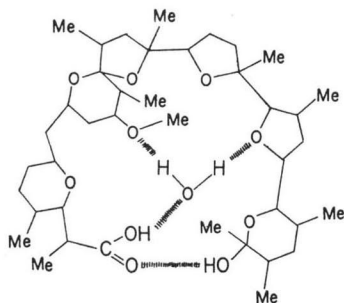
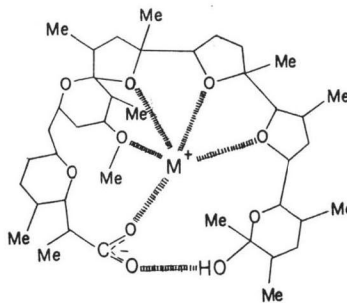
Grisorixin, a polycyclic polyether antibiotic of the nigericin group, showed ionophorous properties. The conformations of crystallized grisorixin and its metallic salts are very similar and are compared in this paper. The physico-chemical properties of the salts are described. The chemical oxidation of grisorixin methyl ester allowed us to isolate several oxidation products whose structures are described.

Grisorixin is a polycyclic polyether ionophorous antibiotic, active against gram-positive bacteria and fungi, and produced by a *Streptomyces griseus*. The production and the structure of this antibiotic are described in the previous paper<sup>1</sup>. In the present paper, we describe the conformation of grisorixin and its metallic salts, and the chemical and spectral study of derivatives we have prepared.

## Structure of Grisorixin and its Monovalent Metal Salts

The structure of crystalline grisorixin monohydrate has been elucidated by ALLEAUME (unpublished data). The molecule takes a cyclic conformation (Fig. 1) similar to those of its  $\text{Ag}^+$  and  $\text{Tl}^+$  salts<sup>2,3</sup> (Fig. 2). In the uncomplexed monohydrate, the water molecule replaces the metal ion, and is hydrogen-bonded to two oxygen atoms and one hydrogen atom of the ligand. The cyclic conformation is further stabilized by an intramolecular hydrogen bond between the carboxyl oxygen and the terminal OH. A study of the effect of dilution on the position of the carbonyl peak of grisorixin in the IR spectrum in  $\text{CHCl}_3$  showed the same small effect previously observed for nigericin and monensin<sup>4</sup>, corroborating the intramolecular

Fig. 1. Conformation of grisorixin monohydrate

Fig. 2. Conformation of grisorixin salts  
M = Ag or Tl

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Table 1. Association constants of monovalent cation salts of grisorixin determined at 25°C in methanol

Cation	Log $K_A$
Li <sup>+</sup>	2.42
Cs <sup>+</sup>	3.19
Na <sup>+</sup>	4.06
Rb <sup>+</sup>	4.10
K <sup>+</sup>	5.24
Tl <sup>+</sup>	5.85
Ag <sup>+</sup>	6.91

nature of the hydrogen bond ( $\nu_{C=O}$ , 1707  $\text{cm}^{-1}$  at  $6.2 \times 10^{-2}$  M;  $\nu_{C=O}$ , 1699  $\text{cm}^{-1}$  at  $6.2 \times 10^{-4}$  M).

We have prepared the Na<sup>+</sup>, K<sup>+</sup>, Ag<sup>+</sup> and Tl<sup>+</sup> crystalline salts of grisorixin. The K<sup>+</sup> and Ag<sup>+</sup> salts are isomorphous. The other monovalent cations—Li<sup>+</sup>, Rb<sup>+</sup>, and Cs<sup>+</sup>—only give amorphous salts obtained as solid forms. The association constants of these salts (Table 1) have been determined by potentiometric and conductometric methods<sup>5)</sup>, and the results ob-

tained show that grisorixin binds strongly to each of these monovalent ions, without, however, a particular selectivity for any one of them.

As in the case for most antibiotics of the nigericin group, complexed salts are formed when the cation is surrounded by the helicoidal chain of the molecule, with the cation linked by coordinate bonds to four oxygen atoms of the polycyclic polyether chain (Fig. 2). The bond between the cation and one of the oxygens of the carboxylate group is clearly shorter than the others, which corroborates the salt-like nature of the complex, as opposed to a mere coordination complex.

The NMR study of the K<sup>+</sup> salt of grisorixin shows the participation of the methoxyl group in cation complexation. This is also true for the Ag<sup>+</sup> and Tl<sup>+</sup> salts. The signal of this methoxyl group is shifted 0.16 ppm downfield compared with grisorixin itself, in acetone-D<sub>6</sub>, and 0.21 ppm downfield compared with grisorixin itself in CCl<sub>4</sub>. Other signals are also shifted in the methyl and methylene regions (Fig. 3).

The mass spectra of the Na<sup>+</sup> and K<sup>+</sup> salts of grisorixin show very different fragmentation processes from those observed in the case of the uncomplexed acid molecule. Most peaks in

Fig. 3. NMR spectrum of grisorixin potassium salt (acetone-D<sub>6</sub>, 100 MHz)

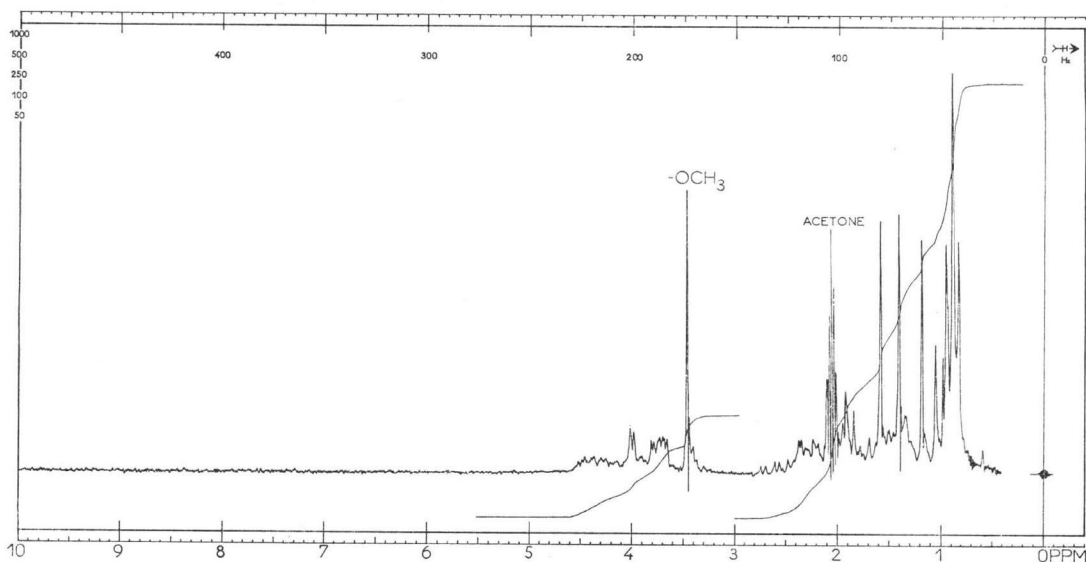


Fig. 4. Mass spectrum of grisorixin potassium salt

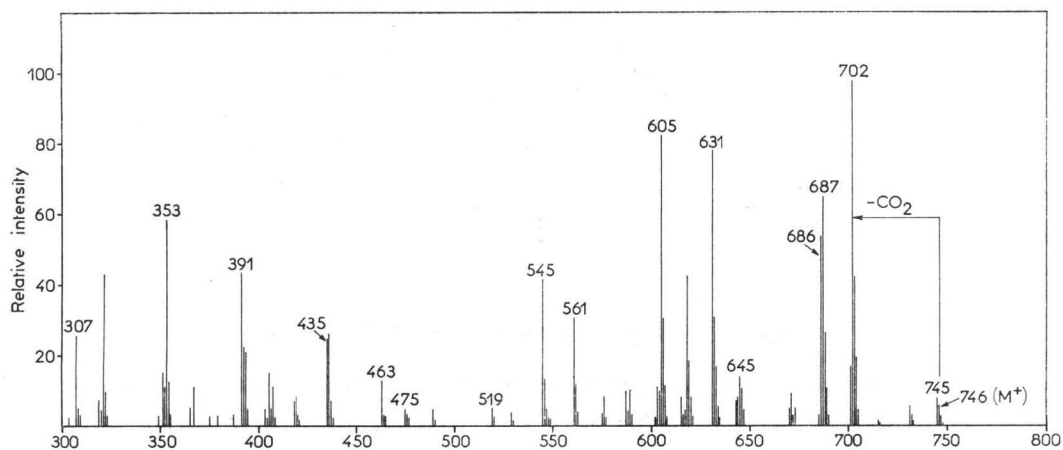
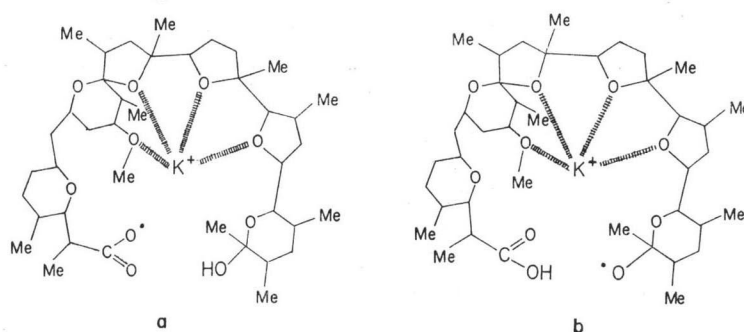
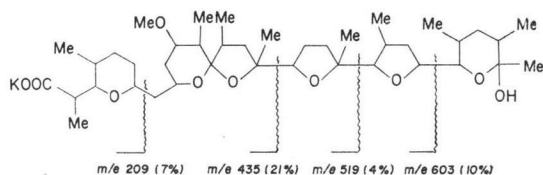
Fig. 5. Structure of the molecular ion *a* or *b*

Fig. 6. Mass spectral fragmentation of grisorixin potassium salt.

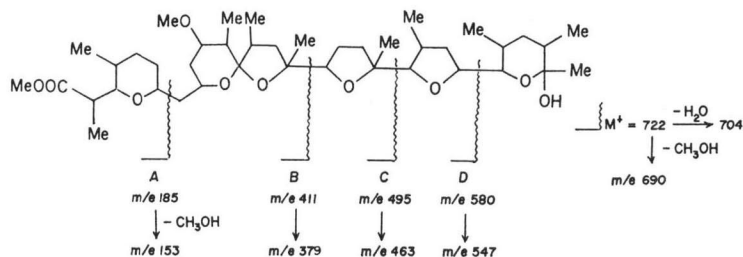


(Fig. 4), the molecular ion peak (*a* or *b*) (Fig. 5) can be seen at  $m/e$  746, as well as a very intense M-1 peak at  $m/e$  745. The base peak occurs at  $m/e$  702, and corresponds to the loss of  $\text{CO}_2$  by the parent ion. This loss of  $\text{CO}_2$  is never seen in the spectra of the uncomplexed antibiotic or its derivatives other than salts. Similar observations have been made by CHAMBERLIN and AGTARAP<sup>6)</sup> for monensin and nigericin. The fragmentation processes described by these authors can be applied to the spectra of the  $\text{Na}^+$  and  $\text{K}^+$  salts of grisorixin, and the cleavage patterns can be explained by analogy to the earlier work.

However, low-intensity peaks appear at  $m/e$  209,  $m/e$  435,  $m/e$  519, and  $m/e$  603 which may be due to cleavage processes similar to those observed in the uncomplexed acid molecule (Fig. 6).

the spectra of the salts are due to fragment ions which contain the alkali metal cation. To account for the formation of such ions, one might first suppose that a molecule of the salt in the gas phase may be represented in the same way as the silver salt (Fig. 2). In the spectrum of the  $\text{K}^+$  salt of grisorixin

Fig. 7. Mass spectral fragmentation of grisorixin methyl ester.



### Chemical Study of the Structure of Grisorixin

We have already described the derivatives prepared by reduction, acetylation, and acid treatment of grisorixin<sup>7)</sup>. The oxidation by CrO<sub>3</sub> of grisorixin methyl ester (Fig. 7) in aqueous acetic acid was carried out at room temperature according to the method described by KUBOTA *et al.*<sup>8)</sup> We have thus obtained several products, which were separated into an acidic fraction and a neutral fraction.

Fig. 7 shows the main fragment ions observed in the mass spectrum of the methyl ester of grisorixin, and which are present in the spectra of the products of chromic acid oxidation.

### Study of the Acidic Fraction

This fraction was treated with diazomethane in ether. Its numerous constituents were separated by column chromatography on silica gel and purified on preparative tlc plates. Five of them have been identified, all methyl diesters.

The first one, with molecular formula C<sub>13</sub>H<sub>22</sub>O<sub>6</sub>, is the same as one of the derivatives obtained by KUBOTA *et al.*<sup>8)</sup> during the chromic acid oxidation of nigericin methyl ester under the same conditions. The mass spectrum of this diester (Fig. 8, compound 1) shows three intense peaks at *m/e* 258 (molecular ion), *m/e* 185 (corresponds to ion A in the spectrum of grisorixin methyl ester), and *m/e* 171 (cleavage of the bond between the tertiary carbon and the THP ring). The signals of the ester methyl groups appear at 3.59 (s) and 3.62 ppm (s) in the NMR spectrum (60 MHz, CCl<sub>4</sub>).

The second diester (Fig. 8, compound 2), with molecular formula C<sub>15</sub>H<sub>26</sub>O<sub>6</sub>, was also isolated by KUBOTA *et al.*<sup>8)</sup> Its IR spectrum (CCl<sub>4</sub>) shows an OH absorption band at 3480 cm<sup>-1</sup> and an intense peak at 1730 cm<sup>-1</sup> due to the ester carbonyls. The ester methyl group peaks in the NMR spectrum (60 MHz, CCl<sub>4</sub>) appear at 3.60 (s) and 3.68 ppm (s). Lastly, the mass spectrum shows peaks at *m/e* 302 (molecular ion), *m/e* 284 (M<sup>+</sup>-H<sub>2</sub>O), *m/e* 185 (ion A), *m/e* 215 and *m/e* 117.

The third diester, with molecular formula C<sub>25</sub>H<sub>42</sub>O<sub>8</sub>, is assigned structure 3 (Fig. 8). Its NMR spectrum (60 MHz, CCl<sub>4</sub>) shows two singlets at 3.68 and 3.71 ppm, due to the ester methyl groups, and one peak at 3.32 ppm which reveals a methoxyl group on one of the spiroketal rings. The molecular ion peak appears at *m/e* 470 in the mass spectrum. Ion A (*m/e* 185→153) may be seen, as well as ion B (*m/e* 411→379), and a peak at *m/e* 285.

The NMR spectrum (60 MHz, CCl<sub>4</sub>) of derivative 4 (Fig. 8), with molecular formula C<sub>34</sub>H<sub>50</sub>O<sub>10</sub>, shows that it is also a methyl diester (singlets at 3.60 and 3.67 ppm), containing a

spiroketal group bearing a methoxyl function singlet at 3.32 ppm). The structure of this derivative is further corroborated by mass spectral peaks at  $m/e$  624 (molecular ion),  $m/e$  593 ( $M^+ - CH_3O^+$ ),  $m/e$  592 ( $M^+ - CH_3OH$ ),  $m/e$  561 ( $M^+ - CH_3O^+$  and  $CH_3OH$ ),  $m/e$  495 (ion C),  $m/e$  411 (ion B), and  $m/e$  185 (ion A).

Lastly, the fifth diester isolated from the esterified acidic fraction is assigned structure **5** (Fig. 8). Only the last ring was oxidized. Its molecular formula is  $C_{41}H_{88}O_{11}$ , and its mass spectrum shows ions A ( $m/e$  185), B ( $m/e$  411), and C ( $m/e$  495), and a peak at  $m/e$  579 corresponding to ion D without transfer of a hydrogen, contrary to the grisorixin methyl ester spectrum.\* The ester methyl group peaks in the NMR spectrum (60 MHz,  $CCl_4$ ) appear at 3.62 (s) and 3.68 ppm (s).

#### Study of the Neutral Fraction

The constituents of this fraction were separated by column chromatography on silica gel and purified on preparative tlc plates. Many derivatives were obtained, from which only two have thus far been identified.

The first is methyl ester **6** (Fig. 8), with molecular formula  $C_{28}H_{46}O_8$ . Its NMR spectrum (60 MHz,  $CCl_4$ ) shows a singlet at 3.33 ppm due to the methoxyl group, a singlet at 3.72 ppm due to a methyl ester, and a 3-proton peak at 2.14 ppm (s) due to a  $CH_3-CO-$  group. The IR spectrum (KBr) shows three carbonyl peaks at 1750, 1740, and  $1725\text{ cm}^{-1}$ . The main peaks of the mass spectrum correspond to ions A, B, and ( $M^+ - CH_3OH$ ).

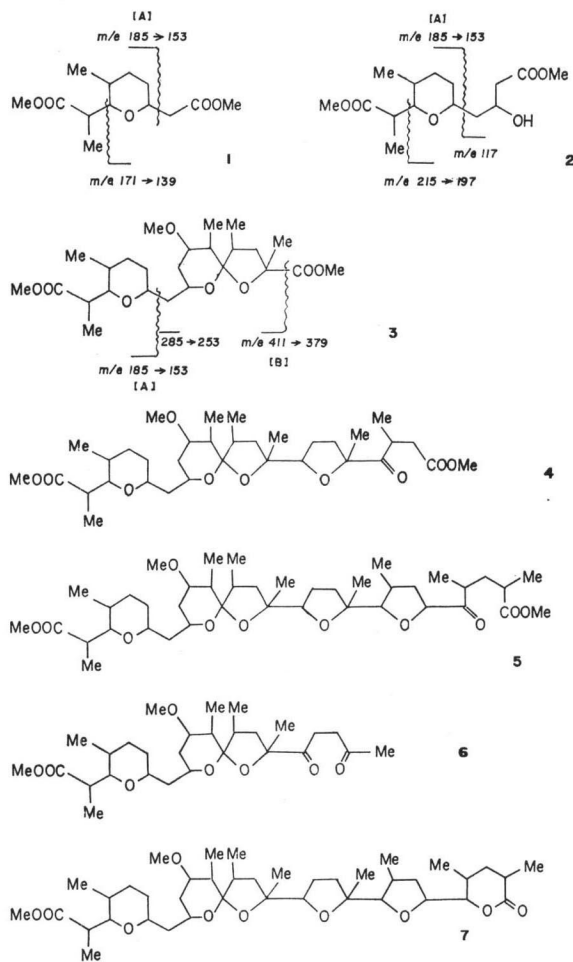
The lactone **7** (Fig. 8), with molecular formula  $C_{40}H_{66}O_{10}$ , is similar to that prepared by KUBOTA *et al.*<sup>9)</sup> by potassium metaperiodate oxidation of nigericin methyl ester. The mass spectrum shows ions A ( $m/e$  185), B ( $m/e$  411), C ( $m/e$  495), and D ( $m/e$  579). The lactone carbonyl band appears at  $1738\text{ cm}^{-1}$  in the IR spectrum (KBr).

#### Experimental

##### General

Melting points (mp) were determined with a Reichert microscope and are uncorrected. Nmr spectra were determined with a Varian Associates HA-100 spectrometer and mass spectra

Fig. 8. Chromic acid oxidation products of grisorixin methyl ester.



\* Note that the proton transfer was not observed in the mass spectrum of nigericin methyl ester, in which ion D appears at  $m/e$  579.

were taken with a Varian MAT CH 5 spectrometer at 70 eV. Column chromatography was performed on Merck silica gel (0.063~0.200 mm). Analytical tlc plates were Merck (silica gel 60) and preparative tlc plates were prepared with Merck silica gel (PF<sub>254+360</sub>).

#### Preparation of the metallic salts of grisorixin

**Sodium salt:** To a solution of 300 mg of grisorixin in 20 ml of ethanol-water (1 : 1) was added 0.1 N NaOH to pH 8.5 (pH meter). This mixture was evaporated under vacuum to dryness. The white residue obtained was dissolved in 10 ml of methanol, and water added until slightly turbid. After several hours at room temperature, white crystals of the sodium salt precipitated. After filtration, the sodium salt was recrystallized from methanol-water as above. Recrystallized sodium salt (280 mg) of grisorixin was obtained mp: 242~246°C.

Calcd. for C<sub>40</sub>H<sub>67</sub>O<sub>10</sub>Na: C 65.73, H 9.24, Na 3.15.

Found: C 65.76, H 9.49, Na 3.18.

**Potassium salt:** This salt was obtained in the same manner as above and was recrystallized twice. Thus, 500 mg grisorixin afforded 450 mg of recrystallized potassium salt, mp: 168~172°C.

Calcd. for C<sub>40</sub>H<sub>67</sub>O<sub>10</sub>K: C 64.31, H 9.04, K 5.23.

Found: C 64.21, H 9.12, K 5.06.

**Silver salt:** To a solution of 330 mg of the sodium salt in 20 ml of aqueous ethanol (1 : 1) was added a solution of 80 mg AgNO<sub>3</sub> in 1 ml of water. After evaporation at room temperature in darkness, crystals of the Ag salt precipitated. These crystals were filtered and recrystallized twice from acetone-water (1 : 1) after standing several hours at room temperature in darkness for the Ag<sup>+</sup> salt is light-sensitive. Recrystallized Ag salt (300 mg) was obtained, mp: 147~153°C.

Calcd. for C<sub>40</sub>H<sub>67</sub>O<sub>10</sub>Ag: C 58.89, H 8.28, Ag 13.22.

Found: C 59.08, H 8.25, Ag 12.83.

**Thallium salt:** To a solution of 300 mg of Ag salt in 20 ml of methanol-water (1 : 1) was added 100 mg of thallium chloride dissolved in a minimal amount of water. After 10 minutes, the precipitate of AgCl was filtered and the mixture left at room temperature for several hours. Crystals of the Tl salt precipitated and were recrystallized from acetone-water (1 : 1) by evaporation at room temperature. Thallium (300 mg) salt was obtained.

Calcd. for C<sub>40</sub>H<sub>67</sub>O<sub>10</sub>Tl: C 52.66, H 7.40.

Found: C 52.86, H 7.58.

#### Chromic oxidation of grisorixin methyl ester

To a solution of 4.25 g of grisorixin methyl ester in 55 ml of glacial acetic acid was added slowly, with cooling and stirring, a solution of 5 g CrO<sub>3</sub> in 60 ml of acetic acid-water (3 : 1). This reaction mixture was left 16 hours at room temperature, diluted with 1,000 ml ice-water, and extracted with chloroform. The chloroform extracts were dried with Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum. The residue was dissolved in ether, extracted with aqueous NaOH (10 %) and washed with water. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under vacuum; 2.5 g of neutral oxidation products were obtained. The basic solution was acidified with 1 N HCl and extracted with ether. This organic phase was washed twice with water and dried (Na<sub>2</sub>SO<sub>4</sub>). After evaporation under vacuum, 1.6 g of acidic oxidation products were isolated and treated with diazomethane in ether.

**Acidic fraction:** The numerous constituents of this fraction were first roughly separated on a silica gel column using an ether-petroleum ether mixture (30 : 70) as eluant. The pure products were then obtained by rechromatographing the fractions three times on preparative tlc plates with the above developing solvent.

Fifteen mg of the dimethyl ester **1** were obtained as an oil of Rf 0.60.

Calcd. for C<sub>13</sub>H<sub>22</sub>O<sub>3</sub>: C 60.45, H 8.58, O 30.97.

Found: C 61.00, H 8.70, O 30.38.

Thirty five mg of **2** were obtained as an oil of Rf 0.35.

Calcd. for  $C_{15}H_{20}O_8$ : C 59.58, H 8.67, O 31.75.

Found: C 59.18, H 8.28, O 32.02.

Thirty five mg of **3** were isolated as an amorphous form of Rf 0.55.

Calcd. for  $C_{25}H_{42}O_8$ : C 63.81, H 8.99, O 27.20.

Found: C 63.85, H 9.15, O 26.97.

Twenty mg of **4** were obtained as a white solid form of Rf 0.75.

Calcd. for  $C_{34}H_{56}O_{10}$ : C 65.36, H 9.03, O 25.61.

Found: C 64.88, H 9.21, O 26.05.

Forty five mg of **5** were isolated as a white solid form of Rf 0.18.

Calcd. for  $C_{41}H_{88}O_{11}$ : C 66.82, H 9.30, O 23.88.

Found: C 66.26, H 9.05, O 24.06.

Neutral fraction: The neutral oxidation products were first chromatographed on a silica gel column using an ether-petroleum ether mixture (65 : 35) and purified twice by preparative silica gel tlc using the same solvent.

Eighty mg of the methyl ester **6** were obtained as an amorphous solid form, Rf 0.55.

Calcd. for  $C_{23}H_{40}O_8$ : C 65.86, H 9.08, O 25.06.

Found: C 65.35, H 9.22, O 25.41.

Forty mg of the lactone **7** were obtained as amorphous form, Rf 0.65.

Calcd. for  $C_{40}H_{86}O_{10}$ : C 67.96, H 9.41.

Found: C 67.30, H 9.32.

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